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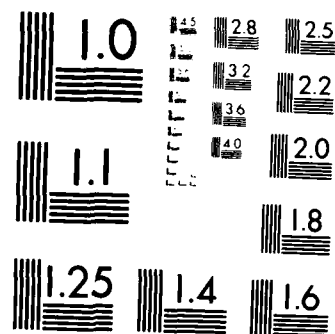
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The Use of ATP-MgCl<sub>2</sub> in the Treatment of  
Injury and Shock

AD-A151 839

Annual Report

Arthur E. Baue, M.D.  
Irshad H. Chaudry, Ph.D.

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Studies have shown that reticuloendothelial (RE) function was significantly depressed following hepatic ischemia and that infusion of ATP-MgCl <sub>2</sub> following ischemia corrected the impairment in RE function. The potential for administration of ATP-MgCl <sub>2</sub> as passive therapy at a time of marked RE depression following ischemia suggests a new therapeutic modality in the treatment of hepatic ischemia. Our results have also shown that infusion of adenine nucleotides-MgCl <sub>2</sub> following severe renal insult significantly improves renal (continued)			

function and suggests that these agents may effectively accelerate the recovery from acute renal failure. Moreover, our results indicate that the accelerated recovery of renal function by ATP-MgCl<sub>2</sub> was concentration dependent and that optimal effects were observed with 50 moles of ATP-50 moles of MgCl<sub>2</sub>. These observations have important implications for future use in organ preservation and management of post-ischemic renal failure. Studies with sepsis have shown that hepatic function was impaired during sepsis and that impaired liver function during sepsis may not only have significance in terms of host defense against bacteremia but may also be associated with pulmonary changes which jeopardize the animals as well. Another study indicated that administration of hypertonic glucose may play some role in the restoration of RE function following sepsis; however, for complete restoration of RE function, the combined usage of ATP-MgCl<sub>2</sub> plus glucose was essential. Studies with splenectomy have shown that splenectomy may not only have deleterious effects in terms of host defense systems, but may also cause prolonged pulmonary changes which may jeopardize the animal as well. Moreover, the results indicated that the spleen plays an important role in the survival of animals following sepsis. Another set of studies indicated that increased metabolic demand following trauma coupled with severe caloric deprivation, may have an effect on RE function and that altered hepatic, pulmonary and RE action may play a role in the mortality of such animals. Studies with prostaglandin indicated that prostaglandins play a protective role during hemorrhage and early sepsis. In another area of research we found that hemorrhagic shock, per se, did not produce any major alterations in red blood cell cations and that the changes that had been reported previously during shock are due to transfusion of stored blood. Our preliminary studies have also indicated that the sodium-potassium pump may also be the carrier for transporting ATP to the cell. Another set of experiments indicated that a conspicuous advantage of positive end-expiratory pressure was observed over zero end-expiratory pressure in experimentally induced pseudomonas pneumonia. This effect was observed not only in terms of improved cardiopulmonary function but also in terms of early survival. Moreover, the results indicated that one of the mechanisms by which positive end-expiratory pressure improves the survival of dogs with pseudomonas pneumonia may be an improvement in pulmonary macrophage function. In another set of preliminary studies, our results indicated that sepsis per se did not alter oxygen consumption at the tissue level and that addition of ATP-MgCl<sub>2</sub> to the incubation medium decreased tissue oxygen consumption by approximately 50%.

## FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (CHEW Publication No. (NIH) 78-23, Revised 1978).

Recent progress can best be summarized by citing the publications from our laboratory supported by the previous year's contract:

1. Chaudry, I.H., Hirasawa, H. and Baue, A.E. "Impairment of reticuloendothelial system function with sepsis and its improvement with ATP-MgCl<sub>2</sub> and glucose administration" *Advances in Shock Research* (Eds: Schumer, W., Spitzer, J.J. and Marshall, B.E.) Alan R. Liss, New York 2:153-162, 1979.
2. Barash, P.G., Burke, M.D., Tilson, M.D., Katz, J.E., Cronan, L.H. and Baue, A.E. "The salutary effects of positive end-expiratory pressure (PEEP) in experimentally induced *Pseudomonas pneumonia*" *Anesthesia and Analgesia* 58:203-215, 1979.
3. Carlson, R.D., Chaudry, I.H. and Baue A.E. "Prostaglandin (PG) metabolism, synthesis, and blockade in hemorrhage and sepsis". *Surg Forum* (in press).
4. Chaudry, I.H., Tabata, Y., Schleck, S. and Baue, A.E. "Impairment of reticuloendothelial function following hepatic ischemia and its restoration with ATP-MgCl<sub>2</sub> administration" *Adv Shock Res* (in press).
5. Chase, H.M., Reynolds, H.Y., Tilson, M.D., Matthay, R.A. and Baue, A.E. "An effect of positive end-expiratory pressure (PEEP) on pulmonary alveolar macrophage function" *Surg Forum* (in press).
6. Chaudry, I.H. and Baue, A.E. "Symposium - New Concepts in Shock Therapy. The use of substrates and energy in the treatment of shock" *Circ Shock* (in press).
7. Baue, A.E. and Chaudry, I.H. "Symposium - New Concepts in Shock Therapy. Some clinical adventures and misadventures" *Circ Shock* (in press).
8. Chaudry, I.H., Tabata, Y., Schleck, S. and Baue, A.E. "Effect of splenectomy on reticuloendothelial (RE) function and survival following sepsis" 39th Annual Mtg. Amer. Assoc. for Surgery of Trauma, Paper 28, Chicago, 1979 (Abstract).
9. Chaudry, I.H., Schleck, S. and Baue, A.E. "Effect of hypertonic solutions on reticuloendothelial (RE) function during sepsis" *Fed Proc* 38 (3), 1193, 1979 (Abstract).
10. Baue, A.E., Schleck, S. and Chaudry, I.H. "Alterations in hepatic function during sepsis" *Fed Proc* 38 (3), 1260, 1979 (Abstract).
11. Chaudry, I.H., Tabata, Y., Schleck, S. and Baue, A.E. "Impairment of reticuloendothelial (RE) function following hepatic ischemia and its restoration with ATP-MgCl<sub>2</sub> administration. *Circ Shock* 6:166, 1979 (Abstract).
12. Kreis, D.J., Baue, A.E. and Chaudry, I.H. "The cause of red blood cell sodium and potassium alterations in shock" *Physiologists* (in press) Abstract.

The following papers which were in press have now been published. They are:

1. Chaudry, I.H., Wichterman, K. and Baue, A.E. "Effect of sepsis on tissue adenine nucleotide levels" *Surgery* 85:205-211, 1979.
2. Lytton, B., Glazier, W.B., Chaudry, I.H. and Baue, A.E. "The use of ATP-MgCl<sub>2</sub> in the treatment of post-ischemic renal injury" *Trans. Amer. Assn. Genito-Urinary Surg.* 70:145-147, 1979.
3. Wichterman, K., Chaudry, I.H. and Baue, A.E. "Studies on the peripheral glucose uptake during sepsis" *Arch Surg* 114:740-645, 1979.
4. Hirasawa, H., Chaudry, I.H. and Baue, A.E. "Beneficial effects of ATP-MgCl<sub>2</sub>-glucose administration on survival following sepsis" *Surg Forum* 29:11-14, 1978.
5. Glazier, W.B., Chaudry, I.H., Siegel, N.J., Kashgarian, M., Lytton, B., and Baue, A.E. "Enhanced recovery from severe ischemic renal injury with ATP-MgCl<sub>2</sub> administration after the insult" *Surg Forum* 29:82-84, 1978.
6. Chaudry, I.H. and Baue, A.E. "Transport of ATP in tissue cells" *Sixth International Biophys Congress, Kyoto, Japan P361, 1978 (Abstract).*

A number of papers are being prepared for submission for publication, but are not cited now because they have not been completed. Also, we have participated in a number of programs in which the work by this contract has been presented. These include participation and presentation of our work at the Eastern Pennsylvania Chapter of the College of Surgeons, Tottstown, PA; Symposium on Vascular Surgery, Montefiore Medical Center, Bronx, NY; Westchester Surgical Society and Providence Surgical Society Meeting at Yale University, New Haven, CT; Surgical Forum Meeting in Los Angeles, CA; New Jersey Medical Society Annual Meeting in Atlantic City, NJ; being a moderator at the Symposium "New Concepts in Shock Therpay" at Williamsburg, VA and presentation of our work at the International Biophysical Congress Meeting in Kyoto, Japan in September, 1978; American College of Surgeons Meeting in San Francisco, CA, in October 1978; Federation of American Society for Experimental Biology in Dallas, TX in April, 1979; the Second Annual Shock Society Meeting in Williamsburg, VA in June, 1979; and the American Association for the Surgery of Trauma Meeting in Chicago, IL in September 1979. In addition, several lectures as Visiting Professor were presented at the following institutions: New England Deaconess Hospital, Boston, MA; Pennsylvania State Medical Center, Hershey, PA; Cleveland Clinic, Cleveland, OH; Northwestern University, Chicago, IL; and various other lectures at regional and local programs on shock and circulatory failure.



The principal findings of the past year will now be summarized:

## 1. Hepatic Ischemia Studies

### A. Reticuloendothelial (RE) Function Following Hepatic Ischemia and Its Restoration with ATP-MgCl<sub>2</sub> Administration.

Recently studies from our laboratory have shown that infusion of ATP-MgCl<sub>2</sub> following 60 minutes of hepatic ischemia proved beneficial for the survival of animals. However, it is not known whether the depression in RE function following hepatic ischemia is affected by administration of ATP-MgCl<sub>2</sub>. To determine this, total hepatic ischemia was produced in rats by ligation of the hepatic artery, portal vein, and the common bile duct. Splenectomy was performed following which a temporary extra corporeal portafemoral shunt was established and maintained throughout the ischemia. At the end of the ischemic period (60 minutes), the ligature was removed, re-establishing blood flow to the liver. The animals then received IV either 0.25ml saline (non-treated) or 0.25ml ATP-MgCl<sub>2</sub> (12.5μmoles each)(treated). Three hours following the end of the ischemia, RE function was evaluated by measuring the intravascular clearance of a <sup>131</sup>I-triolein labelled gelatinized test lipid emulsion. The intravascular half-time (t/2)(mean ± S.E. of eight animals in each group) in sham operated, non-treated and treated animals was 13.5 ± 0.6, 21.4 ± 2.0 and 13.2 ± 0.6 minutes, respectively. Since the t/2 in the non-treated animals was increased approximately by 60%, it indicates the significant depression in RE function was evident even at 3 hours after ischemia. Administration of ATP-MgCl<sub>2</sub> following hepatic ischemia, however, resulted in t/2 values similar to sham-operated animals, indicating that ischemia was reversed with ATP-MgCl<sub>2</sub>. Further studies will help to determine the mechanism by which ATP-MgCl<sub>2</sub> restores the depressed RE function.

## 2. Renal Ischemia Studies

### A. Accelerated Recovery of Acute Renal Failure by Infusion of Adenine Nucleotides - MgCl<sub>2</sub>.

Previous studies have failed to demonstrate that any agent which is given after an acute renal injury can effectively modify the recovery process. We have recently shown that ATP-MgCl<sub>2</sub> infusion following the ischemic insult will successfully ameliorate the recovery of post-ischemic acute renal failure. In the present study, other adenine nucleotides (ADP and AMP) together with MgCl<sub>2</sub> were infused after 30 minutes of bilateral renal artery occlusion. Twenty-four hours later: 1) rats that had received no infusion, ADP alone or only MgCl<sub>2</sub> had reduced GFR (355 ± 40 l/min/100gm B.W. vs 917 ± 36, control, p 0.01), FE<sub>Na</sub> (0.65 ± 0.10% vs 0.17 ± 0.04 for control, p 0.01), decreased RBF (3550 ± 205 l/min/100gm B.W. vs 5095 ± 171 control value), and diminished Uosm (862 ± 110 vs 1425 ± 132 control value; 2) rats given dopamine or phenoxybenzamine maintained low GFR (365 ± 50) despite improved RBF (4678 ± 222); 3) rats infused with either ADP- or AMP-MgCl<sub>2</sub> had marked improved GFR (596 ± 46, p 0.01), increased RBF (4269 ± 223, p 0.01), normalized FE<sub>Na</sub> (0.18 ± 0.07%, p 0.01) and improved Uosm (1201 ± 106, p 0.05). In animals given no

infusion or only  $MgCl_2$ , ultrastructural studies demonstrated focal cellular necrosis and marked generalized tubular cell and mitochondrial swelling, whereas rats infused with ATP- $MgCl_2$  had fewer ultrastructural changes with better preservation of cellular morphology.

The data indicate that adenine nucleotides (ATP, ADP, or AMP) together with  $MgCl_2$  when infused after an acute renal insult significantly improved both glomerular and tubular function and suggest that these agents may effectively accelerate the recovery following acute renal failure.

#### B. Dose Response Relationship of ATP- $MgCl_2$ Administration After the Ischemic Insult.

Renal ischemia is a common and important clinical problem which may result from a period of hypotension due to shock or local vascular occlusion. As a result of a clearer understanding of some of the mechanisms involved in ischemia renal failure, a number of agents have been given to experimental animals after a period of renal ischemia to ameliorate the subsequent effects of injury. Previous work from our laboratory has shown that infusion of 12.5  $\mu$ moles of ATP- $MgCl_2$  after 30 minutes of renal ischemia would markedly accelerate the recovery from this renal injury. This study was designed to determine whether infusion of higher concentrations of ATP- $MgCl_2$  would be more successful in ameliorating a severe renal insult.

Male Sprague Dowley rats (200-300gm) were subjected to 30 minutes of renal ischemia by placing a clamp across the aorta proximal to the left artery and a sling around the right renal artery. After removing the vascular clamp, one group received no infusion and the other groups were given either 12.5  $\mu$ moles, 25  $\mu$ moles, or 50  $\mu$ moles of ATP together with equimolar amounts of  $MgCl_2$ , IV over 10 minutes. The results indicated that the accelerated renal recovery by ATP- $MgCl_2$  was concentration dependent and that optimal effects were observed with 50  $\mu$ moles of ATP-50  $\mu$ moles of  $MgCl_2$ .

### 3. Sepsis-Peritonitis Studies

#### A. Alterations in Hepatic Function During Sepsis.

This study was undertaken to determine whether functional alterations in the liver occur during sepsis. Sepsis in fasted (24 hours) Holtzman rats was produced by cecal ligation and puncture. Saline (3ml/100gm B.W.) was given subcutaneously at that time. Sixteen hours later, the gangrenous cecum was removed; the peritoneal cavity irrigated with warm saline and the abdominal incision closed. Three hours following cecal removal, blood was obtained from the descending aorta and the serum separated. The results (IU/ml, mean  $\pm$  S.E. of eight animals in each group) indicated that during sepsis serum GOT increased from  $37 \pm 2.4$  to  $132 \pm 14.5$ , GPT  $12 \pm 1.9$  to  $42 \pm 3.4$  and alkaline phosphatase  $33 \pm 1.9$  to  $69 \pm 6.1$ . Blood glucose levels decreased from 125mg% to approximately 30mg%. Since the level of the above enzymes increased and since severe hypoglycemia was observed, it suggests that liver function was impaired during sepsis.

Another set of experiments indicated that reticuloendothelial function was impaired and that increased pulmonary localization of the test lipid emulsion occurred during sepsis. Increased pulmonary localization of the lipid emulsion may be due to decreased blood flow to the liver, pulmonary capillary damage or deficiency of circulating opsonic activity. The present results suggest that impaired liver function during sepsis may not only have significance in terms of host defense against bacteremia but may also be associated with pulmonary changes which jeopardize the animal as well.

#### B. Effect of Hypertonic Solution on RES Function During Sepsis.

Recent studies from our laboratory have shown that RES function was depressed during peritonitis and that administration of ATP-MgCl<sub>2</sub> + 50% glucose restored the RES function. Since the treatment solution was hypoertonic and hypertonicity may produce a transient effect on blood flow which may affect the recovery of the RES function, further studies were conducted to determine the role of hypertonicity on the RES function. Peritonitis in fasted rats was produced by cecal ligation and cecal puncture. Saline (3cc/100gm B.W.) was given subcutaneously following cecal ligation and puncture. Sixteen hours following cecal ligation and puncture, the peritoneal cavity was reopened and the gangrenous cecum was removed; the peritoneal cavity was irrigated with warm saline and the abdomen was closed in layers. After measuring blood pressure, animals which were normotensive received intravenously either: 1) 3ml of 50% mannitol; 2) 3ml of 50% glucose; 3) 3ml saline or 4) 0.75ml ATP-MgCl<sub>2</sub> (100 moles + 50 moles) + 2.25ml saline. Two hours following the removal of the cecum, RES function was evaluated by measuring the intravascular clearance of a <sup>131</sup>I-triolein-labelled gelatinized test lipid emulsion.

The intravascular half time (t/2) (Mean + S.E. of 8 animals in each group) in control, mannitol-treated and glucose-treated animals was 7.6 + 0.6, 13.5 + 1.3 and 9.9 + 0.04, respectively. The t/2 in saline-treated (non-treated) or ATP-MgCl<sub>2</sub> alone-treated animals was 12.7 + 1.8 and 9.8 + 0.5, respectively. Since the t/2 in the mannitol-treated animals was approximately doubled (p 0.02) as compared to controls, it indicates that infusion of hypertonic mannitol had no significant effect on the restoration of RES function. Infusion of glucose alone or ATP-MgCl<sub>2</sub> alone, however, decreased the t/2 but these values were still much higher than the group received ATP-MgCl<sub>2</sub> + glucose (t/2=7.1). Previous studies from our laboratory have shown that IV infusion of ATP-MgCl<sub>2</sub> + glucose (but not glucose alone or ATP-MgCl<sub>2</sub> alone) following sepsis had a salutary effect on the survival of animals. The present results indicate that whereas hypertonic glucose or ATP-MgCl<sub>2</sub> alone did decrease the t/2 of the test lipid emulsion, the clearance values were still higher than controls. Thus, it appears that administration of hypertonic glucose may play some role in the resotration of RES function, however, for complete restoration of the RES function the combined usage of ATP-MgCl<sub>2</sub> + glucose was essential.

#### 4. Splenectomy Studies

##### A. Effect of Splenectomy on Hepatic and Lung Uptake of Test Lipid Emulsion.

The results mentioned above have shown that reticuloendothelial (RE) function was depressed following hepatic ischemia and that infusion of ATP-MgCl<sub>2</sub> following ischemia restored the RE function. However, the intravascular half-time ( $t/2$ ) in these sham-operated animals was  $12.8 \pm 0.8$  minutes, which was considerably higher than the values we have obtained previously ( $7.6 \pm 0.6$  minutes). The only difference between the present sham-operated animals and those previously studied was that splenectomy was performed on these animals. Four hours following splenectomy, RE function was evaluated by measuring the intravascular clearance of <sup>131</sup>I-triolein labelled gelatinized test lipid emulsion. In addition, the distribution of the lipid emulsion in liver and lung was determined. The results indicated that splenectomy by itself decreased the hepatic uptake of lipid emulsion by 43%, increased the lung retention by 360% and increased the  $t/2$  by 68%. The mechanism by which splenectomy decreases the hepatic lipid emulsion uptake, increases lung uptake and increases the  $t/2$  in sham-operated animals is not known. Nonetheless, the present experiments suggest that splenectomy may not only have deleterious effects in terms of host defense system but may also cause pulmonary changes which jeopardize the animal as well.

##### B. Time Course of RE Depression Following Splenectomy.

The spleen alone or in combination with other viscera, is the most frequently injured organ following blunt trauma to the abdomen. Splenectomy has usually been the recommended treatment regardless of the type or extent of splenic injury. It is well known that splenectomy causes immunological impairment and increases susceptibility to infection, however, the time course of RE depression as well as alterations in other organ functions following splenectomy is not known. To determine this, rats were splenectomized and RE function evaluated at various intervals following splenectomy and by measuring the intravascular clearance of <sup>131</sup>I-triolein gelatinized lipid emulsion. The intravascular clearance ( $t/2$ , minutes) in sham-operated rats was  $7.6 \pm 0.6$ . The  $t/2$  at 1, 2, 4, 8, 22, 46 and 66 hours following splenectomy was  $6.2 \pm 0.3$ ,  $7.8 \pm 0.8$ ,  $12.8 \pm 0.8$ ,  $9.6 \pm 0.6$ ,  $8.0 \pm 0.5$ ,  $6.9 \pm 0.6$ , and  $8.6 \pm 0.7$ , respectively. The liver and lung % uptake of the injected emulsion in sham-operated animals was  $51.3 \pm 1.8$  and  $1.8 \pm 0.1$ , respectively. Hepatic uptake was  $51.4 \pm 2.0$ ,  $39.2 \pm 4.4$ ,  $27.8 \pm 3.4$ ,  $40.9 \pm 1.6$ ,  $34.4 \pm 2.2$ ,  $46.6 \pm 4.1$  and  $44.8 \pm 2.6$ , respectively at the above mentioned intervals following splenectomy. The corresponding lung uptake was  $2.7 \pm 0.7$ ,  $3.0 \pm 0.9$ ,  $3.0 \pm 0.6$ ,  $14.7 \pm 2.2$ ,  $20.8 \pm 3.6$ ,  $8.0 \pm 1.6$  and  $8.9 \pm 2.0$ , respectively. These results indicate that there is a marked depression in the RE function 4 hours following splenectomy. This was reflected by increased  $t/2$ , decreased hepatic and increased lung uptake of the emulsion. Twenty-two hours after splenectomy, although the  $t/2$  appeared normal, the lung uptake increased by 1058% and hepatic uptake was 33% lower than controls. Forty-six hours following splenectomy the lung uptake was still 344% higher even though hepatic uptake was normal. These results suggest that

splenectomy may not only have deleterious effects in terms of host defense systems but also cause prolonged pulmonary changes which may jeopardize the animal as well.

### C. Effect of Splenectomy on Survival Following Sepsis.

Since the studies described above indicated that RE function in sham-operated animals was decreased following splenectomy, we have studied the effect of splenectomy on the survival of animals following sepsis. Sepsis in splenectomized rats was produced by cecal ligation and puncture. Saline (3ml/100gm B.W.) was given subcutaneously at that time. Ten or 16 hours (early or late sepsis, respectively) following cecal ligation and puncture, the peritoneal cavity was irrigated with warm saline and the abdominal incision closed. Saline (3.75ml) was given intravenously at that time and survival was measured over a period of 5 days. The results indicated that all non-splenectomized early septic rats survived the septic insult. However, the mortality rate was 57.1% in splenectomized animals subjected to early sepsis. Likewise, the mortality rate in non-splenectomized late septic rats was 55% and following splenectomy, it increased to 85%. Thus, splenectomizing the animals prior to cecal ligation and puncture increases the mortality of animals. These results suggest that the spleen plays an important role in the survival of animals following sepsis.

### 5. Effect of Severe Caloric Deprivation on Survival Following Trauma

This study is designed to determine the effects of severe caloric deprivation on various parameters and on the survival of animals following trauma. Rats were fasted for 5-1/2 days (water allowed ad lib) after which a 3 cm midline incision was made and the cecum ligated. Saline (3ml/100gm B.W.) was given S.W. at that time and again at 10, 17, and 34 hours following ligation (1, 1.5 and 2.5ml/100gm BW, respectively). Food was allowed 36 hours after cecal ligation (CL) and survival was measured over a period of five days. The mortality rate in these animals was 60% (12/20) compared to 0% (0/15) in twenty-four hours fasted animals. Starvation alone for seven days did not produce any mortality. In an additional study, reticuloendothelial (RE) function was evaluated 10 hours following cecal ligation by measuring the intravascular clearance of <sup>131</sup>I-triolein labelled gelatinized lipid emulsion. There was no significant difference in the clearance rates of emulsion in various groups suggesting that the phagocytic function was not impaired by starvation plus cecal ligation. However, hepatic and splenic uptake of emulsion decreased by 26% and 74%, respectively following starvation plus cecal ligation and associated with this was 738% increase in retention of the emulsion by the lung. The hematocrit in these animals was 61% (normal value 46). The serum levels of  $\gamma$ -globulin (gm %) in 24 hours fasted, six days fasted and six days starved plus cecal ligated rats were:  $0.49 \pm 0.02$ ,  $0.90 \pm 0.08$  and  $0.33 \pm 0.04$ , respectively. The finding of increased  $\gamma$ -globulin levels during starvation was quite unexpected. Serum GOT levels were not affected by prolonged starvation alone, however, starvation plus cecal ligation results in its elevation  $31 \pm 9$  vs  $85 \pm 10$  IU/ml. Preliminary experiments indicate that hepatic and renal ATP levels decreased from  $2.6 \pm 0.5$  and  $1.8 \pm 0.4$  to  $1.6 \pm 0.1$  and  $1.2 \pm 0.2$   $\mu$ moles/g,

respectively, following starvation plus cecal ligation. These results indicate that increased metabolic demand following trauma (i.e., cecal ligation), coupled with severe caloric deprivation may have an effect on RE function since splenic lipid emulsion uptake decreased following starvation plus cecal ligation. The relationship of these findings to immune function remains to be determined. Although the precise cause of mortality is not known, altered hepatic, pulmonary and RE action may play a role.

#### 6. Prostaglandin (PG) Metabolism Synthesis and Blockade in Hemorrhage and Sepsis.

Studies of *in vivo* PG metabolism by the lung and the effects of PG synthesis blockade by indomethacin (INDO) on survival following hemorrhage and sepsis have been conducted. Pulmonary PG metabolism as assessed by bolus IV injections of 1.24 C <sup>3</sup>H-PGE<sub>2</sub> in awake restrained rats, followed immediately by withdrawal of blood from the subclavian artery for 20 seconds. PGE<sub>2</sub> and metabolites were separated using thin layer chromatography and quantified as fractional radioactivity due to each compound. Unhemorrhaged controls (N=6) showed 79.3% metabolism of injected PGE<sub>2</sub>. Animals subjected to 75 minutes of hemorrhagic at 40mmHg (N=9) showed a decrease in PGE<sub>2</sub> metabolism to 42.6% (p 0.01). Also noted was a decrease in the formation of 15-keto-PGE<sub>2</sub> (28.9% vs 17.3% of the total recovered radioactivity, p 0.05). Whether the decreased metabolism is related to altered transport, decreased degradation of PG or altered pulmonary circulation is not known. PG levels during shock were measured but were so variable as to make interpretations difficult. In another study, IV INDO (5mg/kg) or buffer was given 20 minutes prior to bleeding and INDO (3.5mg/kg/hr) or buffer infused at 0.01ml/min during the 75 minute intervals of hypotension at 40mmHg. Survival (measured over a period of two days) decreased from 68.8% (11/16) in controls to 29.8% (5/17) following INDO treatment (p 0.025). Thus, PGs appear to play a protective role during hemorrhage. In a model of sepsis in rats produced by cecal ligation and punctured followed by cecal excision at 10 or 16 hours (early or late sepsis, respectively), the effect of INDO treatment on survival was studied. INDO treatment comprised: 1mg/kg s.q. twice a day for 72 hrs starting 24 hrs before cecal ligation and puncture; 4mg/kg IP 30 minutes before cecal ligation and puncture and 6.7mg/kg IV at the time of cecal excision. Controls received equal volumes of vehicle. Survival (measured over a period of five days) following early sepsis was 100% (12/12) in controls and 25% (3/12) in INDO treated group (p 0.001). Following late sepsis, survival was 30.8% (4/13) in controls and 23.1% (3/13) in the treated group. It is concluded that PGs play a protective role during early sepsis but that other factors may become more important as sepsis evolves.

#### 7. Factors Affecting Red Blood Cell (RBC) Sodium and Potassium Levels During Shock.

Increases in RBC Na<sup>+</sup> have been observed in several pathological states, including uremia burns, trauma and hemorrhagic shock. Our previous preliminary experiments had shown that RBC Na<sup>+</sup> levels increased while K<sup>+</sup> levels decreased during hemorrhagic shock. However, it was

subsequently determined there was a malfunction in the atomic absorption spectrophotometer which we had been using. Because of this, serious questions are in order concerning the validity of the values obtained as a result of usage of that particular spectrophotometer. In view of this situation, we have re-investigated the effects of hemorrhagic shock and corrective therapy with whole blood transfusions on RBC  $\text{Na}^+$  and  $\text{K}^+$  levels.  $\text{Na}^+$  and  $\text{K}^+$  measurements were performed on a different and functioning spectrophotometer.

Rats were bled rapidly to a pressure of 40mmHg following which no further blood was removed or returned (Group 1). The blood pressure of these animals increased to approximately 70mmHg within 30 minutes of hemorrhage and remained at that level for 2 hours. Another group of animals were bled to 40mmHg and maintained at that level for 1-1/2 hours by transfusion with rats whole blood which had been stored in ACD buffer at 4°C for 6 days and warmed to ambient temperatures prior to administration (Group 2). The volume of the blood transfused per animals equaled 50% of the maximally shed blood volume. The results of eight animals in each group in mM/L were as follows:

	<u>GROUP 1</u>				<u>GROUP 2</u>				<u>STORED BLOOD</u>	
	<u>Control</u>		<u>Shock</u>		<u>Control</u>		<u>Shock</u>			
$\text{Na}^+$	3.7	0.1	3.4	0.1	3.5	0.1	8.4	0.7	8.2	1.4
$\text{K}^+$	99.1	1.9	97.2	1.4	100.3	2.3	85.0	2.1	79.4	8.3

These results indicate that hemorrhagic shock without transfusion therapy does not cause any changes in RBC  $\text{Na}^+$  and  $\text{K}^+$  levels. However, reciprocal  $\text{Na}^+$  and  $\text{K}^+$  alterations in RBC  $\text{Na}^+$  and  $\text{K}^+$  levels. Another set of experiments indicated that the increase in RBC  $\text{Na}^+$  in Group 2 rats in shock transfused with stored blood for 3 days in ACD was 62%, compared to 140% increase following transfusion of 6 days stored blood. RBC  $\text{Na}^+$  of the stored blood itself increased by 75% at 3 days and 127% after 6 days storage at 4°C, probably due to inhibition of the  $\text{Na}^+$  pump at lower temperatures. These results indicate that hemorrhagic shock per se does not produce any alterations in RBC cations. Because storage of blood at low temperatures causes RBC cations to be altered, this suggests that during shock the changes which have been reported previously are due to transfusion of stored blood.

In another set of experiments, sepsis in rats was produced by cecal ligation and puncture as we have described previously. Measurement of RBC  $\text{Na}^+$  and  $\text{K}^+$  levels at 10 and 16 hours (early or late sepsis, respectively) following cecal ligation indicated that there were no significant alterations in RBC cations during early or late sepsis as well.

#### 8. Characterization of a Probably ATP Transport System.

Since our pioneer studies concerning ATP transport into the cell have now been well documented by other investigators, including investigators from the N.I.H., we have begun conducting studies which we hope will

characterize the system which transports ATP into the cell. Our preliminary results have shown that addition of  $10^{-3}M$  ouabain to the incubation medium inhibited ATP uptake process by 70%; moreover, insulin has been shown to stimulate the Na, K-ATPase activity stimulated ATP uptake process by more than 60%. These preliminary results strongly suggest that ATP could be transported into the cell by the sodium pump, i.e., the Na, K-ATPase since the ATP uptake process responded both to the stimulatory effects of insulin and inhibitory effects of ouabain.

Our preliminary results have also shown that various metabolic poisons (e.g. silver nitrate, copper sulphate, zinc acetate, sodium salicylate and dinitrophenol) also inhibited the ATP uptake process. These results may very well indicate that ATP uptake is an active process and that it requires mitochondrially produced ATP for its operation.

#### 9. Indocyanine Green Clearance Studies.

Recent studies from our laboratory have shown that serum GOT and GPT levels were significantly increased following hepatic ischemia and that these levels were significantly decreased following administration of ATP-MgCl<sub>2</sub>. However, we do not know whether hepatic function is altered much earlier than serum enzyme levels and whether ATP-MgCl<sub>2</sub> has any effect on it. We have tested the clearance of indocyanine green in normal rats and the t/2 values were found to be approximately 3.6 minutes. Since our ultimate purpose is to determine the indocyanine green clearance following hepatic ischemia and treatment with ATP-MgCl<sub>2</sub> and since such animals would have to be splenectomized, we have initially studied the effect of splenectomy on the clearance of indocyanine green. These clearances were measured at 0,2,3,4 hours and 1,2,3,6 days following splenectomy. The results indicated that the clearance rate and, therefore, hepatic function was not affected immediately following splenectomy or even after a prolonged period of time such as six days.

#### 10. Lung Studies.

##### A. The Salutary Effects of Positive End-Expiratory Pressure (PEEP) in Experimentally-Induced Pseudomonas Pneumonia.

Controlled data on the effects of positive end-expiratory pressure (PEEP) in the presence of gram-negative pneumonia are unavailable. The present study was carried out to examine the cardiopulmonary response to PEEP in dogs before and after the intratracheal inoculation of an inoculum of Pseudomonas aeruginosa ( $1 \times 10^9$  organisms/kg), thereby allowing us not only to study the cardiopulmonary response to PEEP in the presence of gram-negative pneumonia, but also the effect of PEEP on the course of the experimentally induced pneumonia. Sixteen mongrel dogs were anesthetized (pentobarbital, pancuronium), intubated and ventilated (16ml/kg and 10 respirations/min) for 24 hours with 50% O<sub>2</sub> and 50% N<sub>2</sub>. Half of the animals were maintained with zero end-expiratory pressure (ZEEP) and the remainder had PEEP = 10cm H<sub>2</sub>O. Half of the dogs in each group were challenged with live Pseudomonas and the following were measured (0,1,2,4,8,12,16, and 24 hrs): blood pressure, pulse rate, pulmonary artery pressure, pulmonary capillary wedge pressure, respiratory rate,



effective compliance minute ventilation, tidal volume, pH,  $\text{PaO}_2$ ,  $\text{PaCO}_2$ ,  $\text{PvO}_2$ , cardiac output, and hematocrit.

Three of four infected ZEEP dogs died before 24 hours; all infected animals treated with PEEP and control dogs survived. The infected ZEEP dogs developed a significantly ( $p < 0.05$ ) elevated cardiac index as early as 4 hours, accompanied by a significant increase in venous admixture; oxygenation and compliance deteriorated profoundly. Infected ZEEP dogs also showed signs of early capillary leak with a 50% increase in mean hematocrit, despite the infusion of 63% more fluid (13.3ml/kg/hr) than the control group to maintain a pulmonary capillary wedge pressure of 8mmHg. Cardiovascular and respiratory function in the infected PEEP group was not markedly different from the noninfected PEEP or ZEEP control animals.

The results indicate a conspicuous advantage of PEEP over ZEEP in experimentally induced *Pseudomonas pneumonia*, not only in terms of improved cardiopulmonary function, but also in terms of early survival.

#### B. An Effect of Positive End-Expiratory Pressure (PEEP) on Pulmonary Alveolar Macrophage Function.

Recent studies have shown that positive end-expiratory pressure (PEEP) improves survival in a canine model of *Pseudomonas pneumonia*. Among possible explanations for the observed reduction in mortality, PEEP may improve the function of pulmonary alveolar macrophages (PAMs). This study was conducted to test PAM function after PEEP with an *in vitro* system for quantitating the uptake of  $\text{C}^{14}$ -labelled *Pseudomonas* organisms by PAMs in tissue culture.

Five one-year-old farm-bred beagle dogs were anesthetized (Pentobarbital) and intubated with a double-lumen Carlen endotracheal tube. The lungs were ventilated synchronously (TV 10 cc/kg x 10 min); and a PEEP valve (10cm  $\text{H}_2\text{O}$ ) was used randomly in one circuit or the other so that one lung had PEEP and the other had zero-end-expiratory pressure (ZEEP). After 6 hours the PAMs were lavaged from each lung, quantified, assessed for viability (dye exclusion), and cultured as a monolayer. *Pseudomonas* organisms were grown in and cultured as a monolayer. *Pseudomonas* organisms were grown in broth containing  $\text{C}^{14}$ -labeled amino acids. Viable radioactive organisms were counted, opsonized (Ig G antibody) and added to the PAM monolayer for 30 min in a ratio of 100 bacterial per cell. Bacterial uptake was expressed as CPM per lysed monolayer. The results indicated that equal numbers of PAM were obtained from PEEP and ZEEP lungs and the viability index was also equal. Bacterial uptake was linear over the first 45 min by both ZEEP and PEEP PAMs (with  $r = .98$  and  $.99$ , respectively); but the slope of uptake was increased 40% in the PEEP PAMs. The mean CPM was increased 50% for the PEEP PAMs at 30 min ( $p < 0.05$  by paired and unpaired t-test) and 29% at 45 min ( $t < 0.05$  by pair t-test).

These results suggest that one of the mechanisms by which PEEP improves the survival of dogs with *Pseudomonas pneumonia* may be an improvement in pulmonary macrophage function.

### 11. Effect of ATP-MgCl<sub>2</sub> on Tissue Oxygen Consumption

To determine whether ATP-MgCl<sub>2</sub> has any effect on tissue oxygen consumption, rat liver slices (0.2-0.5mm thick) were intubated at 37°C in 7.0ml of Krebs-Ringers Phosphate buffer in a sealed chamber containing Clarke's oxygen electrode. The slices were incubated initially for 5 min to obtain temperature equilibration. Glucose (10mM) was then added and oxygen consumption was followed for a period of ten minutes. Following this, ATP-MgCl<sub>2</sub> (5mM) was added and the effect of the nucleotide on tissue oxygen consumption was determined.

Preliminary experimentation indicates that oxygen consumption in normal tissues was 60.4 moles O<sub>2</sub>/min/gm of tissue and in tissues from septic animals was 66.5 moles O<sub>2</sub>/min/gm of tissue. Thus, sepsis per se did not alter oxygen consumption at the tissue level. Preliminary experiments also indicate that addition of 5mM of ATP-MgCl<sub>2</sub> to the incubation medium decreased tissue oxygen consumption by approximately 50%.

Studies in progress which we hope to complete in the remaining period of the present contract year.

1. Measurement of Hepatic Function Following Hepatic Ischemia.

We have tested the clearance of indocyanine green in normal animals and have determined that splenectomy by itself does not affect the clearance rate of indocyanine green. We are now in the process of setting up experiments to determine the clearance of indocyanine green following hepatic ischemia. Our previous results have shown that serum GOT and GPT levels significantly increased following hepatic ischemia and that the level of these enzymes significantly decreased following administration of ATP-MgCl<sub>2</sub>. The purpose of the present experiments would, therefore, be to determine if hepatic function is also beneficially affected by treatment with ATP-MgCl<sub>2</sub> as were the serum enzyme levels. It is quite possible that hepatic function is altered much earlier than serum enzyme levels following treatment with ATP-MgCl<sub>2</sub>.

2. Effect of ATP-MgCl<sub>2</sub> on Release of Intracellular Enzymes from Tissues of Animals in Shock.

Serum enzyme measurements have been made extensively in diagnosis for more than two decades. However, the mechanism by which intracellular enzymes are released into the circulation from the damaged cells remains unknown. Complete destruction of the cell with necrosis leads to the discharge of its contents but little is known of the cause of the increased membrane permeability in reversible states of shock, trauma, or inflammation. It has been suggested that membrane permeability is linked to cellular metabolism and it has been shown that enzyme leak from intact muscle into the medium was increased by anoxia, glucose deprivation, exposure to excessive potassium ions or treatment with metabolic inhibitors. Furthermore, it has been suggested that the integrity of cell membrane as assessed by its ability to prevent the leakage of enzymes depends on the energy content of the cell, a decrease of which may be a common factor in clinical situations associated with elevated serum enzyme activities.

We have previously shown that ATP levels of various tissues decreased during shock and that infusion of ATP-MgCl<sub>2</sub> restored the cellular nucleotides and proved beneficial in the treatment of shock. It is quite possible that ATP-MgCl<sub>2</sub> protects leakage of intracellular enzymes from cells during shock and that some of the beneficial effects of ATP-MgCl<sub>2</sub> were by the above mechanism. We plan to explore this possibility.

3. Hepatic Sodium-Potassium Transport in Adrenalectomized Animals During Shock

Previous work from our laboratory has shown that sodium-potassium transport in the liver is adversely affected during hemorrhagic shock and that the membrane-bound sodium-potassium transport mechanism, and not membrane permeability, was directly affected under those conditions. We now plan to determine whether the alterations in hepatic sodium-potassium transport during shock were mediated by the product(s) of the adrenal gland. In order to study this, bilateral adrenalectomy will be performed

on Albino Holtzman rats four to five days prior to the study and these animals will subsequently be provided with corticosteroid replacement prior to producing hemorrhagic shock. Hemorrhagic shock will then be produced and hepatic sodium-potassium levels will be measured as we have done previously. If there are no alterations in hepatic sodium-potassium levels in such animals, this will indicate that the product(s) of the adrenal glands were responsible for altering the hepatic sodium-potassium transport capability during shock. Following the completion of these experiments, we plan to infuse catecholamines, steroids, aldosterone, or a combination of the above to determine if we can adversely affect the hepatic sodium-potassium transport capability in normal rats. These experiments will help to determine whether or not the product(s) of the adrenal gland are responsible for producing sodium-potassium alterations in shock. Following the completion of the experiments we plan to infuse catecholamines, steroids, aldosterone or a combination of the above to determine if we can adversely affect the hepatic sodium-potassium transport capability in normal rats. These experiments will help to determine whether or not the product(s) of the adrenal gland are responsible for producing sodium-potassium alterations in shock.

#### 4. Mechanism of Adenine Nucleotide Translocation During Shock

Previous studies from our laboratory have shown that ATP is capable of crossing the intact cell membrane of tissues obtained from normal animals and that this process is enhanced during shock. Moreover, our studies have shown that the transport of ATP into cells could be a carrier-mediated process. We now plan to study the mechanism of ATP translocation during control conditions and determine if this process is affected during shock. For these studies, isolated plasma membrane and sarcoplasmic reticulum from muscle of control and shock animals will be used.

#### 5. Splenic Autotransplantation Studies

Recent studies from our laboratory have shown that splenectomy may not only have deleterious effects in terms of host defense systems but may also cause prolonged pulmonary changes which jeopardize the animal as well. We now plan to autotransplant approximately 25% of the spleen following splenectomy and determine whether the alterations in RE function and lung lipid emulsion retention could be prevented by this procedure.

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